

NIGELLA SATIVA AND ALLIUM SATIVUM IMPROVES MASH BEAN SEEDS DURING STORAGE

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ABSTRACT

Mash bean [*Vigna mungo* (L.) Hepper] seeds were collected from different shops of Pakistan, for analyzing mycoflora by using ISTA (International Seed Testing Association) techniques namely blotter, agar plate and deep-freezing methods. Nineteen fungal species belonging to 10 genera were isolated from mash beans while agar plate method was better for the isolation of seed borne fungi followed by blotter method and deep freezing methods. *Aspergillus flavus* followed by *Aspergillus niger*, *Aspergillus wentii* and *Penicillium* spp. were the most dominant fungi. During storage, growth of saprophytic fungi increased and the growth of pathogenic fungi also increased a little at room temperature. Surface sterilization with 1% calcium hypochlorite greatly reduced the infestation of fungi. *Aspergillus flavus* was found to be more prevalent during storage. *Nigella sativa* and *Allium sativum* controlled the incidence of pathogenic fungi as well as saprophytic fungi at different time interval and temperatures (4°C and room temperature (27 ± 2°C)). Storage at low temperature was better to reduce the fungi on mashbean seeds.

Key words: ISTA techniques, low temperature, storage fungi, surface sterilization.

INTRODUCTION

Mash bean [*Vigna mungo* (L.) Hepper] is an important legume crop widely grown in Asia. Its growth can be observed both in summer as well as in winter. It is a cheap source of protein in human diet. It is very nutritious as it contains high levels of protein (25g/100g), potassium (983 mg/100g), calcium (138 mg/100g), iron (7.57 mg/100g), niacin (1.447 mg/100g), thiamine (0.273 mg/100g), and riboflavin (0.254 mg/100g) (Sharma *et al.*, 2012). Javaid *et al.* (2005) reported four fungal species namely *Aspergillus niger*, *Aspergillus terreus*, and *Fusarium equiseti* from mash bean where *Aspergillus niger* was most dominant one.

Nigella sativa belongs to Ranunculaceae family, normally develops in Eastern Europe, Middle East, and Western Asia. For many years, these seeds are used for the remedy in many ailments. In Islamic nations, this plant is important due to its numerous useful properties. The greatest part of the remedial properties of this plant is due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil (Ketenoglu *et al.*, 2020). Some minor substances like phenolic compounds, tocopherols and sterols have several beneficial characteristics including antioxidant and anti-inflammatory capabilities (Ketenoglu *et al.*, 2020).

Allium sativum (Garlic) belongs to Alliaceae family grown widely throughout the world. It has many secondary metabolites like alliin, allicin, saponin, rutin, quercetin, oleanolic acid, ferulic acid, chlorogenic acid which are not only important for human health but also inhibit the growth of various microorganisms like bacteria, fungi, protozoa etc. (Ankri and Mirelman, 1999; Lawson, 1998; Block, 2010). Pharmacologically, allicin is the most important and the most active substance and it is found in the fresh extract of *Allium* which significantly reduce seed infection by *Drechslera* on rice and treated seeds had significantly higher viability (Jones *et al.*, 2007).

In view of the economic importance of this crop, present work was carried out to explore the mycoflora associated with mash bean.

MATERIALS AND METHODS

Mash bean seeds were collected from different areas of Pakistan namely Lahore, Thatta, Quetta and Karachi and tested with three methods (Blotter, agar plate and deep freezing methods). In Blotter method, one set of seeds (100 seeds) treated with 1 % calcium hypochlorite for 5 minutes and placed on three layers of moistened blotter paper. The plates were incubated for 7 days at room temperature (27 ± 2°C). Another set of 100 seeds were treated with sterilized distilled water before placing in plates. Same procedure was followed for agar plate method but

instead of blotter paper, seeds were placed on potato dextrose agar (PDA). However in case of deep freezing method, plates after placing on blotter paper, were incubated for 24 hours each at 20°C and -2°C followed by incubation at room temperature $27 \pm 2^\circ\text{C}$ under 12h for 5 days with alternating cycles of light and dark (Anonymous, 1993). Two hundred seeds (hundred for sterilized and hundred for non-sterilized) used in each method with 20 seeds per plate (5 replicate of each method). Fungi growing on mash bean seeds were identified using literature (Barnett, 1960; Booth, 1971; Domsch *et al.*, 1980).

The area containing more fungal infestation on mash bean seeds were selected for another experiment. In this experiment one gram of *Nigella* powder and *Allium sativum* (garlic) powder were added to 100 g of mash bean seeds (1% treatment). Same procedure was applied for treatment with 2 gram and after applying these seeds stored at room temperature and 4 °C for 120 days. The seeds were observed at 0, 20, 40, 80 and 120 days intervals. Mycoflora were detected by using agar plate method (Anonymous, 1993). Non treated seeds served as control.

RESULTS AND DISCUSSION

A total of nineteen fungal species (10 genera) were isolated from mash bean seeds. Among all the three methods, agar plate method yielded maximum number of fungal species (18 species with 10 genera) followed by blotter and deep freezing methods. Among all isolated fungi, *Aspergillus flavus* and *Mucor* sp. were dominant followed by *Fusarium solani* and *Scopulariopsis brevicaulis*. However, *A. niger*, *A. fumigatus*, *F. solani* and *S. brevicaulis* were recorded in all the three methods (Table 1). Hussain *et al.* (2007) suggested agar plate method with PDA to be better than blotter method in terms of percentage recovery of fungi in lentils Niaz and Dawar (2009) reported that deep freezing method was considered best for the isolation of *Drechslera* spp., *Fusarium* spp., and *Penicillium* spp. Surface sterilization has greatly reduced the incidence of saprophytic fungi. Surface sterilization with calcium hypochlorite significantly ($P < 0.01$) reduced the incidence of *Aspergillus flavus*, such as in blotter method incidence of *A. flavus* was reduced from (15.58 ± 4.29) to (8.33 ± 2.43) in sterilized condition. In deep freezing condition *A. flavus* reduced from (11.41 ± 3.67) to (4.58 ± 1.87) . In agar plate method, *A. flavus* also reduced by surface sterilization with 1% calcium hypochlorite from (58.75 ± 7.97) to (32.33 ± 5.57) (Table 1). Surface sterilization has greatly reduced the incidence of saprophytic fungi as well as pathogenic fungi ($P < 0.001$). Similar results were also reported by Tariq *et al.* (2005) on soybean.

Samples from Malir region showed maximum number of fungi so these samples were treated with *Nigella sativa* and *Allium sativum* for the observation of mycoflora using agar plate method. This sample after treatment were stored at 4°C and room temperature ($27 \pm 2^\circ\text{C}$) for 120 days. Eleven fungal species belonging to 7 genera were isolated from mash beans that include *Acremonium* spp., *A. flavus*, *A. niger*, *A. restrictus*, *A. versicolor*, *F. solani*, *Monilia* spp., *Mucor* spp., *Rhizopus* sp. and *S. brevicaulis*. Among all the fungal species, *A. flavus*, *A. fumigatus*, *A. niger*, *Rhizopus* sp., *Penicillium* sp., and *Mucor* sp. appears at all storage days in control samples of mash bean seeds. Various authors reported inhibitory activity of *A. sativum* against mould. It is considered to be due to alliin, thiosulfonates and other compounds showing fungistatic activity against species of *Aspergillus* like *A. niger*, *A. fumigatus*, *A. flavus*, *A. terreus* and *Penicillium* species (Harris *et al.*, 2001; Hafez and Said, 1997; Ankri and Mirelman, 1999). A number of effective biochemical reported from *Nigella sativa* (mainly Thymoquinone (TQ), thymohydroquinone (THQ), didthymoquinone, thymol, carvarol, α - and β -Pinene, d-limonine, d-citranellol, etc) has been reported to be antimicrobial principles in *N. sativa* (mainly TQ) against gram-negative and gram positive bacteria, fungi (TQ), viruses, parasites and Schistosomes (Forouzanfer *et al.*, 2014). Effectiveness of TQ, however, varies depending upon the target species (Forouzanfer *et al.*, 2014). Effect of *Nigella sativa* and *Allium sativum* on fungi was higher on room temperature. *A. flavus*, *A. niger* and *Penicillium* spp. were found to be most dominant throughout the storage period in both temperatures. Both concentrations (1, 2 %) of *N. sativa* and *A. sativum* were helpful in reduction of fungal colonization. It was observed that in control samples, the incidence of fungi was increased as the storage days increased. However, treated mash bean seeds at both temperature reduced or eliminated the colonization of fungi as the storage days increased (Table 2a and 2b).

Present studies showed that temperature factor was effective against the mycoflora as the increase in temperature has increased the incidence of storage fungi. So storage at low temperature can be effective for decreasing the incidence of storage fungi. Proper and long term storage using *A. sativum* and *N. sativa* will be helpful in minimizing the incidence of saprophytic fungi and deterioration of mash bean.

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Table 1. Seed borne fungi of Mash bean seeds collected from different areas of Pakistan.

Fungi	Surface Sterilized				Surface non-sterilized			
	Blotter method	Agar plate method	Deep freezing method	Blotter method	Agar plate method	Deep freezing method	Blotter method	Deep freezing method
<i>Ahluia</i> sp.	NSI	NSI	NSI	NSI	NSI	NSI	NSI	NSI
	0	0	0	0	0	0	0	0
	1% ± SE	1% ± SE	1% ± SE	1% ± SE	1% ± SE	1% ± SE	1% ± SE	1% ± SE
		2.66 ± 0.62	0.16 ± 0.15	0	3.83 ± 0.53	0	0	0
<i>Acremonium</i> sp	0	0	10	5.5 ± 2.3	1	0.66 ± 0.63	0	0
	0	0	10	5.5 ± 2.3	1	0.66 ± 0.63	0	0
	0	0	10	5.5 ± 2.3	1	0.66 ± 0.63	0	0
	0	0	10	5.5 ± 2.3	1	0.66 ± 0.63	0	0
<i>Aspergillus flavus</i>	8	8.33 ± 2.43	12	32.33 ± 5.57	5	4.58 ± 1.87	10	15.58 ± 4.29
	8	8.33 ± 2.43	12	32.33 ± 5.57	5	4.58 ± 1.87	10	15.58 ± 4.29
	8	8.33 ± 2.43	12	32.33 ± 5.57	5	4.58 ± 1.87	10	15.58 ± 4.29
	8	8.33 ± 2.43	12	32.33 ± 5.57	5	4.58 ± 1.87	10	15.58 ± 4.29
<i>Aspergillus fumigatus</i>	2	0.66 ± 0.43	2	1.16 ± 0.75	2	1.91 ± 1.66	2	0.66 ± 0.43
	2	0.66 ± 0.43	2	1.16 ± 0.75	2	1.91 ± 1.66	2	0.66 ± 0.43
	2	0.66 ± 0.43	2	1.16 ± 0.75	2	1.91 ± 1.07	3	1.91 ± 1.66
	2	0.66 ± 0.43	2	1.16 ± 0.75	2	1.91 ± 1.07	3	1.91 ± 1.66
<i>Aspergillus niger</i>	4	4.5 ± 3.55	2	0.66 ± 0.47	3	1.58 ± 0.92	2	0.66 ± 0.43
	4	4.5 ± 3.55	2	0.66 ± 0.47	3	1.58 ± 0.92	2	0.66 ± 0.43
	4	4.5 ± 3.55	2	0.66 ± 0.47	3	1.58 ± 0.92	2	0.66 ± 0.43
	4	4.5 ± 3.55	2	0.66 ± 0.47	3	1.58 ± 0.92	2	0.66 ± 0.43
<i>Aspergillus ochraceus</i>	1	0.5 ± 0.47	1	0.41 ± 0.39	0	0	0	0
	1	0.5 ± 0.47	1	0.41 ± 0.39	0	0	0	0
	1	0.5 ± 0.47	1	0.41 ± 0.39	0	0	0	0
	1	0.5 ± 0.47	1	0.41 ± 0.39	0	0	0	0
<i>Aspergillus parviticus</i>	0	0	1	0.58 ± 0.55	0	0	0	0
	0	0	1	0.58 ± 0.55	0	0	0	0
	0	0	1	0.58 ± 0.55	0	0	0	0
	0	0	1	0.58 ± 0.55	0	0	0	0
<i>Aspergillus restrictus</i>	0	0	0	0	3	1.33 ± 0.95	0	0
	0	0	0	0	3	1.33 ± 0.95	0	0
	0	0	0	0	3	1.33 ± 0.95	0	0
	0	0	0	0	3	1.33 ± 0.95	0	0
<i>Aspergillus versicolor</i>	0	0	2	0.33 ± 0.21	0	0	0	0
	0	0	2	0.33 ± 0.21	0	0	0	0
	0	0	2	0.33 ± 0.21	0	0	0	0
	0	0	2	0.33 ± 0.21	0	0	0	0
<i>Aspergillus wentii</i>	0	0	2	0.83 ± 0.53	0	0	0	0
	0	0	2	0.83 ± 0.53	0	0	0	0
	0	0	2	0.83 ± 0.53	0	0	0	0
	0	0	2	0.83 ± 0.53	0	0	0	0
<i>Drechslera</i> sp	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
<i>Fusarium solani</i>	4	1.58 ± 0.79	6	4 ± 1.48	5	3.08 ± 1.27	8	2.41 ± 0.63
	4	1.58 ± 0.79	6	4 ± 1.48	5	3.08 ± 1.27	8	2.41 ± 0.63
	4	1.58 ± 0.79	6	4 ± 1.48	5	3.08 ± 1.27	8	2.41 ± 0.63
	4	1.58 ± 0.79	6	4 ± 1.48	5	3.08 ± 1.27	8	2.41 ± 0.63
<i>Mucor mucedo</i>	0	0	0	0	0	0	1	0.5 ± 0.47
	0	0	0	0	0	0	1	0.5 ± 0.47
	0	0	0	0	0	0	1	0.5 ± 0.47
	0	0	0	0	0	0	1	0.5 ± 0.47
<i>Mucor piriformis</i>	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
<i>Mucor</i> sp	9	10.75 ± 2.55	8	11.66 ± 2.6	1	2.66 ± 2.55	12	7.58 ± 2.33
	9	10.75 ± 2.55	8	11.66 ± 2.6	1	2.66 ± 2.55	12	7.58 ± 2.33
	9	10.75 ± 2.55	8	11.66 ± 2.6	1	2.66 ± 2.55	12	7.58 ± 2.33
	9	10.75 ± 2.55	8	11.66 ± 2.6	1	2.66 ± 2.55	12	7.58 ± 2.33
<i>Penicillium</i> sp	0	0	1	0.5 ± 0.47	0	0	0	0
	0	0	1	0.5 ± 0.47	0	0	0	0
	0	0	1	0.5 ± 0.47	0	0	0	0
	0	0	1	0.5 ± 0.47	0	0	0	0
<i>Rhizopus stolonifer</i>	2	1.16 ± 0.83	9	22.41 ± 5.07	3	8.91 ± 4.63	3	3.41 ± 1.70
	2	1.16 ± 0.83	9	22.41 ± 5.07	3	8.91 ± 4.63	3	3.41 ± 1.70
	2	1.16 ± 0.83	9	22.41 ± 5.07	3	8.91 ± 4.63	3	3.41 ± 1.70
	2	1.16 ± 0.83	9	22.41 ± 5.07	3	8.91 ± 4.63	3	3.41 ± 1.70
<i>Scolecariopsis brevicornis</i>	3	4.91 ± 2.51	4	1 ± 0.44	2	1.91 ± 1.52	5	3.16 ± 1.4
	3	4.91 ± 2.51	4	1 ± 0.44	2	1.91 ± 1.52	5	3.16 ± 1.4
	3	4.91 ± 2.51	4	1 ± 0.44	2	1.91 ± 1.52	5	3.16 ± 1.4
	3	4.91 ± 2.51	4	1 ± 0.44	2	1.91 ± 1.52	5	3.16 ± 1.4
<i>Trichoderma hamatum</i>	0	0	4	1.33 ± 0.56	0	0	0	0
	0	0	4	1.33 ± 0.56	0	0	0	0
	0	0	4	1.33 ± 0.56	0	0	0	0
	0	0	4	1.33 ± 0.56	0	0	0	0

NSI = Number of sample infested; % = Infection%; S.E = standard error

Table 2(a). Effect on mash bean seeds treated with two concentrations of *Allium sativum* at different temperatures.

Fungi	4°C														
	0%						1%								
Days	0	20	40	80	120	0	20	40	80	120	0	20	40	80	120
<i>Aspergillus flavus</i>	38.33 ± 0.27	3.33 ± 0.27	23.33 ± 0.59	30 ± 0.62	100 ± 0	60 ± 1.24	33.33 ± 0.36	3.33 ± 0.27	0	0	38.33 ± 1.76	0	1.66 ± 0.13	3.33 ± 0.27	25 ± 0.7
<i>Aspergillus fumigatus</i>	8.33 ± 0.36	1.66 ± 0.13	6.66 ± 0.36	10 ± 0.4	16.33 ± 0.39	3.33 ± 0.27	0	0	0	0	0	0	0	0	0
<i>Aspergillus niger</i>	3.33 ± 0.13	6.66 ± 0.27	8.33 ± 0.36	26.66 ± 0.75	0	18.33 ± 0.49	18.33 ± 0.23	0	0	0	41.66 ± 1.44	1.66 ± 0.13	0	0	0
<i>Aspergillus ochraceus</i>	26.66 ± 0.27	16.66 ± 0.82	0	0	0	10 ± 0.81	0	0	0	0	8.33 ± 0.27	0	0	0	0
<i>Aspergillus restrictus</i>	63.33 ± 0.89	28.33 ± 0.13	10 ± 0.81	8.33 ± 0.36	8.33 ± 0.27	0	0	0	0	0	0	0	0	0	0
<i>Fusarium solani</i>	16.66 ± 0.32	16.66 ± 0.39	31.66 ± 0.13	38.33 ± 1.76	45 ± 0.23	0	0	0	0	1.66 ± 0.13	0	0	0	0	0
<i>Mucor sp.</i>	10 ± 0.4	6.66 ± 0.54	8.33 ± 0.56	23.33 ± 0.59	30 ± 0.12	8.33 ± 0.27	5 ± 0.4	0	0	0	0	0	0	0	0
<i>Penicillium sp</i>	11.66 ± 0.27	16.66 ± 0.82	23.33 ± 0.89	26.66 ± 0.75	40.66 ± 1.44	0	0	0	0	0	0	0	0	0	0
<i>Rhizopus sp.</i>	66.66 ± 1.8	56.66 ± 1.9	23.33 ± 0.27	35 ± 0.18	100 ± 0	5 ± 0.4	0	0	0	0	0	0	0	0	0
ROOM TEMPERATURE (27±2°C)															
<i>Acromonium sp.</i>	28.33 ± 0.13	38.33 ± 0.27	33.33 ± 0.36	26.33 ± 0.49	33.5 ± 0.62	3.33 ± 0.27	0	0	0	0	13.33 ± 0.59	0	0	0	0
<i>Aspergillus flavus</i>	55 ± 1.24	59 ± 0.4	28.33 ± 0.13	16.66 ± 0.13	61.66 ± 1.44	45 ± 0.4	20 ± 0.84	31.66 ± 0.27	8.33 ± 0.68	0	31.66 ± 1.42	5 ± 0.4	3.33 ± 0.13	3.33 ± 0.27	0
<i>Aspergillus niger</i>	11.66 ± 0.59	17.66 ± 0.44	33.33 ± 0.49	18.33 ± 0.13	26.66 ± 0.82	31.66 ± 1.34	5 ± 0.4	3.33 ± 0.27	0	0	021.66 ± 0.59	6.66 ± 0.36	0	0	0
<i>Aspergillus ochraceus</i>	10 ± 0.47	5 ± 0.4	20 ± 0.84	0	0	5 ± 0.28	5 ± 0.4	0	0	0	3.33 ± 0.27	1.66 ± 0.13	0	0	0
<i>Fusarium solani</i>	10 ± 0.47	10 ± 0.4	20 ± 0.75	28.33 ± 0.19	0	20 ± 1.63	16.66 ± 0.47	0	0	0	36.66 ± 0.13	0	0	0	0
<i>Mucor sp.</i>	70 ± 1.31	55 ± 0.24	75 ± 0.94	23.33 ± 0.27	100 ± 0	45 ± 1.33	3.33 ± 0.27	3.33 ± 0.22	0	0	10 ± 0.81	5 ± 0.26	0	0	0
<i>Penicillium sp</i>	10 ± 0.4	33.33 ± 1.7	46.66 ± 0.1	75 ± 0.83	100 ± 0	8.33 ± 0.1	35 ± 0.13	0	0	0	11.66 ± 0.27	5 ± 0.23	0	0	0
<i>Rhizopus sp</i>	41.66 ± 1.8	41.66 ± 1.2	50 ± 2.35	100 ± 0	100 ± 0	41.66 ± 0.2	16.66 ± 1.36	0	0	0	5 ± 0.4	0	0	0	0

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